Supporting information

Enhanced Electroactivity and Substrate Affinity of Microperoxidase-11 Attached to Pyrene-linkers π-π Stacked on Carbon Nanostructure Electrodes

K. Sudhakara Prasad, Charuksha Walgama, and Sadagopan Krishnan*
Department of Chemistry, Oklahoma State University, Stillwater, OK, USA- 74078.

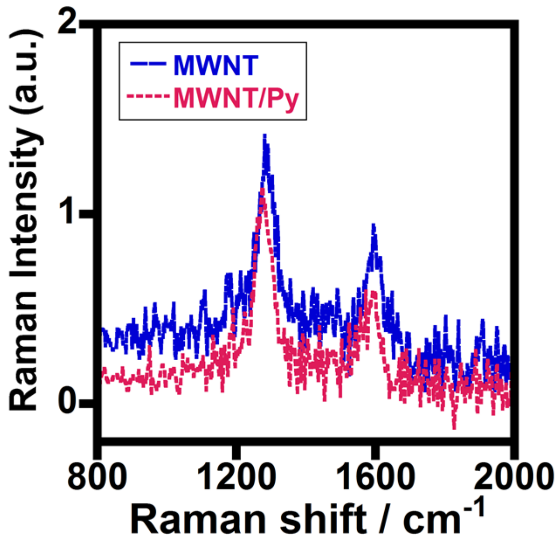

Fig. S1. Raman spectra of MWNT and MWNT/Py films on HPG electrodes.

FTIR Characterization. The HPG electrode and MWNT showed the carbonyl vibration peaks at 1660 cm^{-1} (Figure S2). The FTIR spectra were taken after each modification step on HPG electrodes (Figure S2). MP-11 exhibited peaks between the regions 1500-1600 and 1600-1700 cm^{-1}, which were assigned to the amide-I C=O and the amide-II N-H bending frequencies typical of a peptide backbone.[1-2] The free amine groups of MP-11 vibration bands (Lys-13 and N-terminus Val-11) were assigned at 1627 cm^{-1}. MWNT/Py modified HPG electrodes exhibited bands at 1665 cm^{-1} for carbonyl stretching and a broad band at 3480 cm^{-1} for the –OH stretching, both are attributed to the end –COOH groups of the Py-linker molecules.

The EDC-NHS treatment of MWNT/Py assemblies led to the disappearance of the –OH stretching band of –COOH (originally appeared at 3480 cm^{-1}) due to its conversion to N-succinimidyl groups and the appearance of new peaks centered at 1452 and 1769 cm^{-1} were attributed to C-N and C=O stretchings, respectively.[3,3] The IR-spectrum obtained after the covalent bonding of MP-11 to MWNT/Py assemblies depicted characteristic bands for peptide amide-I and amide-II bonds in the region of 1500 to 1700 cm^{-1}. Additionally the appearance of bands in the region of 3270 to 3400 cm^{-1} represent the amide A and B bonds of MP-11.[2] Taken together, the FTIR characterization confirmed the covalent immobilization of MP-11 to MWNT/Py units on HPG electrodes (Figure S3).

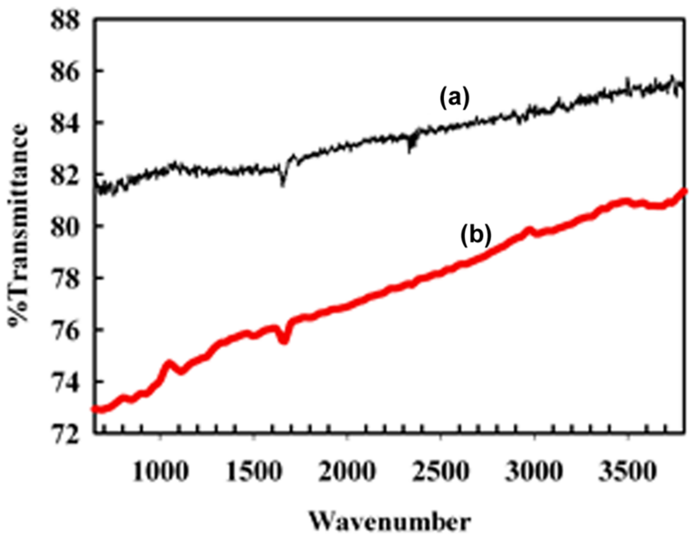

Fig. S2. FTIR spectra of (a) only HPG and (b) only MWNT.

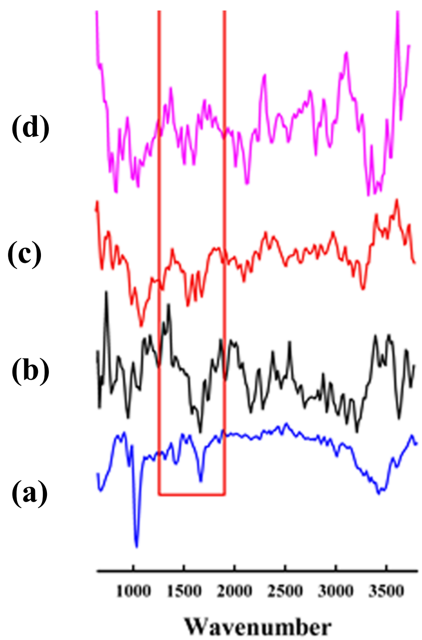

Fig. S3. FTIR spectra of (a) MWNT/Py; (b) EDC-NHS treated MWNT/Py; (c) MP11-amine$_{cov}$ films; and (d) only MP-11.

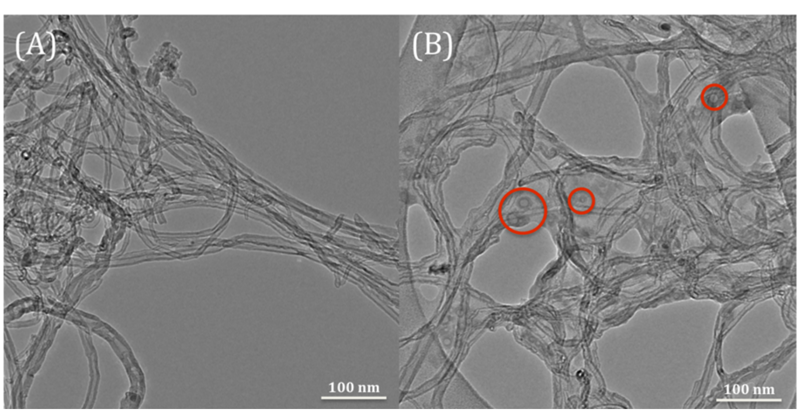

Fig. S4. TEM images for (A) MWNT/Py and (B) MP11-amine$_{cov}$ films (MP-11 features around the tubes are circled).

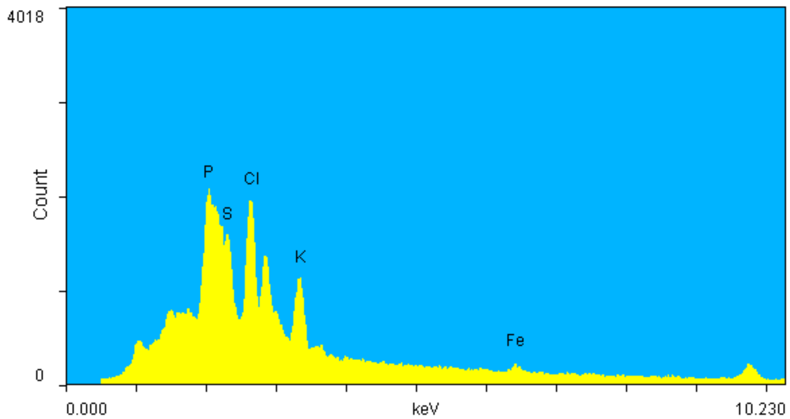

Fig. S5. EDS of the MP11-amine$_{cov}$ film. The presence of P, K, and Cl peaks is attributed to arise from PBS.

Table S1. Comparison of the electroactive MP-11 amounts on MWNT/Py electrodes with control films and the reported myoglobin-amine$_{cov}$ film.

MP-11 Film	Γ / pmol cm^{-2}	Ref.
MP11-amine$_{cov}$	2385 ± 103	This study
HPG/MWNT-MP11	1250 ± 66	
HPG/MP11	619 ± 28	
HPG/Py-MP11	746 ± 30	
myoglobin-amine$_{cov}$	300 ± 60	4

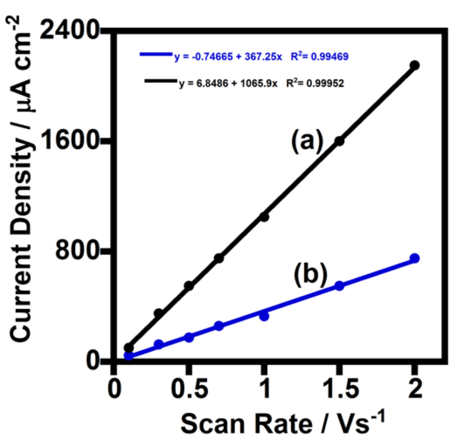

Fig. S6. Current density versus scan rate plot for the charging currents measured for (a) HPG/MWNT/Py electrode and (b) polished HPG electrode in the absence of immobilized MP-11.

Calculation of the direct electron transfer rate constant of MP11-amine$_{cov}$ films.

$$E_{pc} = \left[E^0 - \frac{RT}{\alpha nF}\ln\left(\frac{\alpha nF}{RTk_s}\right)\right] - \frac{RT}{\alpha nF}\ln(\upsilon) \quad (1)$$

$$E_{pa} = \left[E^0 + \frac{RT}{(1-\alpha)nF}\ln\left(\frac{(1-\alpha)nF}{RTk_s}\right)\right] + \frac{RT}{(1-\alpha)nF}\ln(\upsilon) \quad (2)$$

$$\Delta E_p = \frac{2.3RT}{\alpha(1-\alpha)nF}\left[\alpha\log(1-\alpha) + (1-\alpha)log\alpha - logk_s - log\left(\frac{RT}{nF}\right)\right] + \frac{2.3RT}{\alpha(1-\alpha)nF}\log(\upsilon) \qquad (3)$$

Where E^0 is the formal potential, α is the electron-transfer coefficient, k_s is the heterogeneous electron transfer rate constant for a surface confined redox process, υ is the scan rate, and n is the number of electrons transferred (n= 1 for MP11-heme). The average value of the transfer coefficient for the MP11-amine$_{cov}$ film was calculated using the slopes of the plots of E_{pa}, E_{pc}, versus ln(υ) according to Equations 1 and 2. Then Equation 3 was used to plot ΔE_p values against log(υ) to obtain the k_s value (Figure S7).[5]

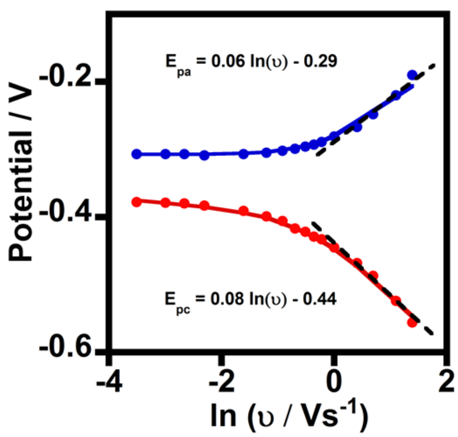

Fig. S7. Relationship between peak potentials (E_p) and natural logarithm of scan rate for MP11-amine$_{cov}$ films at pH 7.4, PBS. The linear fit lines at higher scan rates and the corresponding equations are shown.

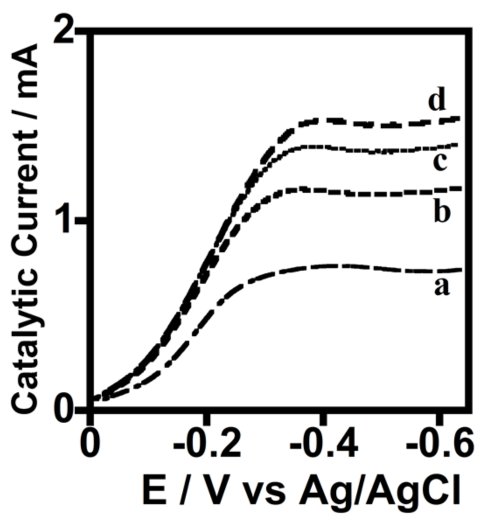

Fig. S8. Reduction currents versus t-BuOOH concentration at 1000 rpm catalyzed by the MP11-amine$_{cov}$ film in pH 7.4 PBS for **a.** 0.8, **b.** 1.6, **c.** 3.2, and **d.** 4.8 mM t-BuOOH.

1 M. F. Cabral, J. D. Barrios, E. M. Kataoka, S. A. S. Machado, E. Carrilho, C. D. Garcia and A. A. Ayon, *Colloids Surf. B,* 2013, **103**, 624– 629.

2 A. Barth, *Prog. Biophys. Mol. Bio*., 2000, **74**, 141-173.

3 F. Zhang, X. Chen, R. A. Boulos, F. M. Yasin, H. Lu, C. Raston and H. Zhang, *Chem.*

Commun., 2013, **49**, 4845-4847.

4 C. Walgama and S. Krishnan, *J. Electrochem. Soc.,* 2014, **161**, H47-H52.

5 S. Ray, S. Chand, Y. Zhang, S. Nussbaum, K. Rajeshwar and R. Perera, *Electrochim. Acta,* 2013, **99**, 85-93.